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Short communication

Thermal inactivation and nucleic acid amplification-based testing for SARS-CoV-2

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1. Introduction

Recently, Pang and colleagues reported that thermal inactivation of clinical samples at +56 °C adversely affected the efficiency of RT-PCR for SARS-CoV-2 detection, likely by disrupting the integrity of the SARS-CoV-2 RNA with subsequent risk of false negative results [1]. However, the impact of heat inactivation on nucleic acid amplification-based testing results remains controversial. Thus, Chen and colleagues reported that the inactivation by incubating sample at +56 °C for 30 min had no significant effect on the detection of SARS-CoV-2 by real-time RT-PCR [2].

In April 2020, Abbott Molecular (Des Plaines, IL, USA) received emergency use authorization (EUA) approval from Food and Drug Administration (FAD), USA, for real-time RT-PCR test to detect SARS-CoV-2 RNA in clinical specimens from suspected COVID-19 patients [3]. The RealTi*me* SARS-CoV-2 assay (Abbott Molecular) amplifies target regions of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes. The assay is run on the automated Abbott m2000 SP/rt platform currently in use internationally by reference laboratories and university hospitals. The RealTi*me* SARS-CoV-2 assay has been validated for clinical use, and with the high-throughput, fully automated Abbott m2000 SP/rt system, this assay will accelerate the pace of SARS-CoV-2 testing [4].

The instructions for use of the RealTi*me* SARS-CoV-2 assay (Abbott Molecular; reference 09N77-095) do not give details on pre-analytical processing of samples, and only claims that all human sourced materials should be considered infectious and be handled using appropriate laboratory biosafety practices [3].

Iorder to provide protection of our laboratory staff, and in the absence of instruction for clinical specimen inactivation from the manufacturer, we investigated herein whether thermal inactivation could affect the results of viral detection using RealTi*me* SARS-CoV-2 assay (Abbott Molecular).

2. Material and methods

Samples from COVID-19 patients hospitalized at the university *Hôpital Européen Georges Pompidou*, Paris, France, were included in the study. All patients had a positive result of SARS-CoV-2 using Allplex™ 2019-nCoV Assay (Seegene, Seoul, Korea), a multiplex real-time PCR assay detecting E, RdRP and N coronavirus target genes.

Study patients were divided into two groups: group I with E, RdRp and N genes C_t (threshold cycle) values above 32.0 and less 37.0 corresponding to low SARS-CoV-2 RNA load ; and group II with E, RdRp and N genes C_t values less than 32.0 corresponding to high SARS-CoV-2 RNA load.

A total of 40 patients were prospectively included, 19 in group I and 21 in group II. All COVID-19 patients were further subjected to nasal swabbing, as described [5]. After sampling, nasal swab was placed in a 15 mL plastic tube containing 3 mL of physiological serum (NaCl 0.9 %).

SARS CoV-2 RNA was further detected in parallel in paired native and heat-inactivated nasal specimen using RealTi*me* SARS-CoV-2 assay (Abbott Molecular) with Abbott m2000 SP/rt platform in a biosafety level (BSL)-2 virology lab, according to manufacturer's instructions. Heat incubation was carried out in water bath at +56 °C for 30 min.

The proportions of patients with positive PCR results by Abbott RealTi*me* SARS-CoV-2 assay using non-inactivated and heat-inactivated samples were compared using the Wilcoxon matched-paired signed rank test. The differences between coronavirus RdRp and N genes C_t values were compared using the paired two-tailed Student's *t* test within each

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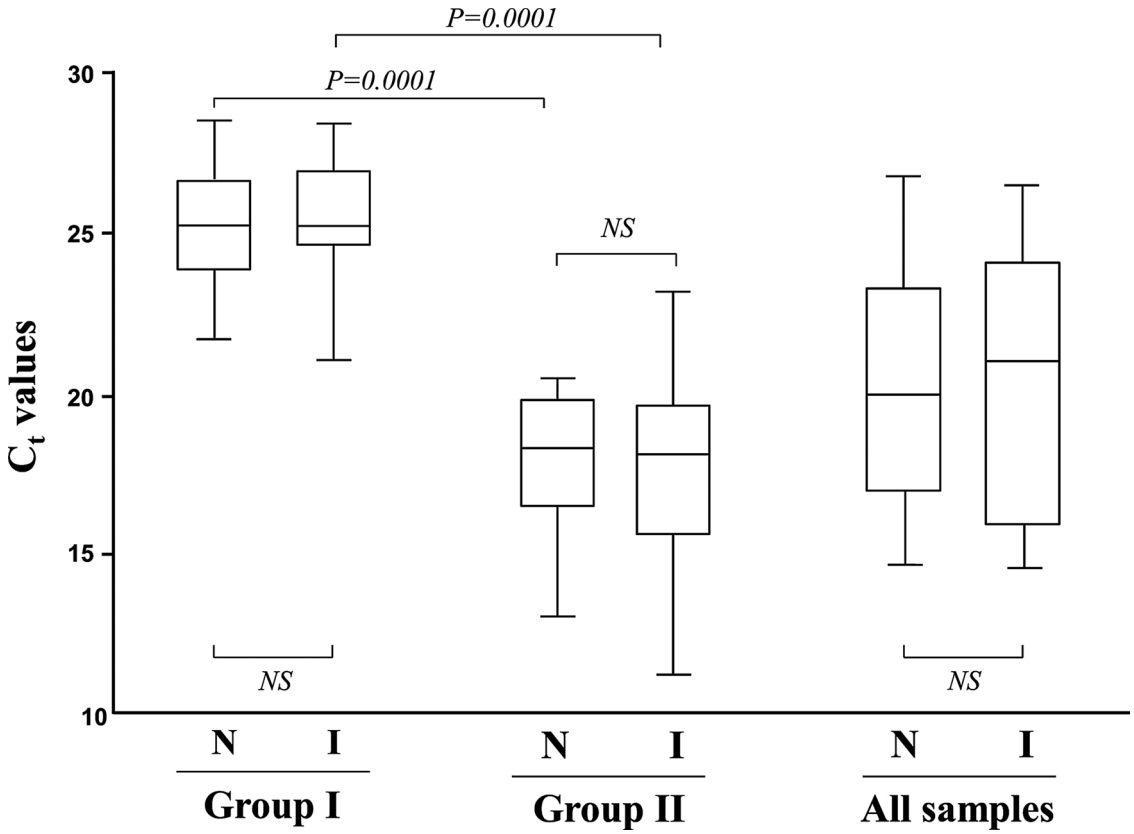

Fig. 1. Distribution of SARS-CoV-2 RdRp and N genes C_t values (in arbitrary unit) of virus detection from non-inactivated (N) and heat-inactivated (I) swab samples by RealTime SARS-CoV-2 assay (Abbott Molecular). For each distribution, the blank box represents the interquartile ranges, the horizontal bar corresponds to the median and the vertical bars and hats indicate the 1st and 3rd quartiles. Comparisons used Student's *t* test for paired data and Welch's *t* test between groups I and II. The 2 samples with high C_t values of group I undetectable after heat-inactivation were excluded from quantitative analysis. NS: Not significant.

group and between groups using the Welch's *t* test assuming unequal variances.

The study was exempted from informed consent application, according to the French public health code for non-interventional study (CSP, article L 1121-1.1; https://www.legifrance.gouv.fr/). Data analyses were carried out using anonymized database.

3. Results

All 40 COVID-19 patients showed SARS-CoV-2 positive Abbott results using non-inactivated nasal swab samples, but only 38 (95 %) positive results [group I: 17/19 (89.5 %); group II: 21/21 (100 %)] using heat-inactivated samples, without statistical difference ($P = 0.219$). The mean (±1 standard deviation) coronavirus genes C_t values of non-inactivated or heat-inactivated samples in group I was higher than those in group II [non-inactivated samples: 25.02 ± 1.81 arbitrary units (au) *versus* 17.83 ± 2.21 au, $P < 0.0001$; and inactivated samples: 25.37±1.76 au *versus* 17.92 ± 3.07 au, $P < 0.0001$]. The mean differences in paired C_t values between non-inactivated and inactivated swab samples showed a slight increase after heat-inactivation (+0.23 ± 1.38, n = 38), without any statistical significance ($P = 0.284$), in group I (+0.36 ± 1.04, n = 17, $P = 0.153$), as well as in group II (+0.11±1.42, n=21, $P = 0.746$). The results are depicted in Fig. 1.

4. Discussion

The World Health Organization stressed the need that health laboratories adhere to strict biosafety practices when testing clinical specimens for SARS-CoV-2 (*5*) [6]. In addition to mandatory personal protective equipment in BSL-2 lab, the issue is still debated of viral inactivation before nucleic acid amplification test. In the present series, heat-inactivation of clinical specimen did not affect significantly the results of SARS-CoV-2 detection, despite very slight increase of C_t values and rare undetectability with few samples harboring low viral load after thermal inactivation.

In conclusion, our observations point that heat-inactivation is a convenient and easy tool to address the biosafety issue when using the FDA-approved RealTi*me* SARS-CoV-2 assay (Abbott Molecular), that should be recommended.

Authors' contributions

All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

J-LB and IP performed the experiments, and collected data. LB, DV, IP and RSMB analyzed the data and generated the figure. HP and IP supervised this study. All authors wrote and edited the manuscript.

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Declaration of Competing Interest

No authors declared any potential conflicts of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104588.

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